Routine Cytological Staining Techniques

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Theoretical Background and Practice

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and

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MACMILLAN

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Preface

We had several reasons for writing this book and several goals in mind when we started our investigations into routine fixation and staining. Above all we wanted to know more about the mechanisms of the staining processes, and to gain an insight into the differences between staining cytological and histological material. We believe that the results of our research are of considerable assistance to anyone involved in routine cytological diagnosis, since the quality of the diagnosis depends on the quality of the smear. We do not consider immunotechniques and enzyme staining to be routine, so these methods are not discussed in our book.

We have learned that when the mechanisms of the staining processes are understood, one can manipulate the staining results according to one's wishes, and standardise the staining. Both goals are most easily reached if the staining solutions are prepared in the laboratory, a process that has proved to be easier than we had anticipated. This manual therefore contains details of methods for preparing staining and fixing solutions, together with practical advice on the most economical methods of staining and fixing.

We also discuss the interpretation of the staining results, and the need to be critical of unnecessary sub-optimal results. We stress that cellular and nuclear dimensions in cytological slides are totally dependent on the cytopreparatory technique employed, and differ significantly from those in tissue sections.

The idea that the quality of the slide is of paramount importance for the success of cytodiagnosis was first realised by one of the authors (M.E.B.) when she was trained in the laboratory of Dr B. Naylor in Ann Arbor, Michigan, during the period 1967–68. In the following years, we noticed at various cytology congresses that many cytological diagnoses were made on sub-optimal slides, and the cytopathologists did not even know exactly how these were fixed and stained. In discussions we became aware that neither we nor our fellow cytologists knew exactly what component in the routine stain stained what in our slides. The explanations we found in cytology and histology books concerning staining cytological slides were not satisfactory for us. We therefore realised that we had to investigate for ourselves in order to find the right answers. Since we thought that knowledge concerning the components of the cell was a keystone in such investigations, we sought contact with the world of biology, and therefore this book is written by a cytologist and a biologist. The very satisfying contacts with other research workers in Europe, Japan and the USA have greatly influenced our insight into this complicated subject. When we had acquired a knowledge of staining mechanisms, we were able to exploit the staining techniques instead of suffering by them, and to profit from their use in our diagnostic work.

It should be clearly understood that our work is far from complete. We hope, however, that this book will enable readers to find the preparation and staining of cytological slides to be as exciting and satisfying as the authors do themselves.

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Many people have given us invaluable advice concerning this book. We thank them all, in particular the following.

Dr R.W. Horobin of the University of Sheffield, UK, read through the first draft of the book and gave us priceless constructive criticism. He also provided us with the data on the physical and chemical characteristics of the dyes, as published in this book. Professor Dr D. Wittekind of the University of Freiburg, West Germany, has shown from the beginning of our work a great interest in our book, and has shared with us the results of his own experiments with the Romanowsky–Giemsa staining method.

We are grateful for the beautiful scanning electron microscope pictures, which form an essential part of the book by showing the three-dimensional aspects of cells in smears. They were provided by Ms P.H.T. van der Zanden and Dr P. Kenemans from the division of gynaecological oncology, University of Nijmegen, The Netherlands.

Last but not least we want to thank the cytotechnologists of the Leiden Cytology Laboratory for their endurance when trying to do cytodiagnostic work on smears stained in all colours of the rainbow, and the cytotechnologists of the SSDZ in Delft for their co-operation in developing various cytopreparatory techniques.

The drawings in this book were made with great enthusiasm by B. Brest from the Technical University in Delft. The cover is based on the colours of stained cells in cytological slides. Our good friend Frans Rombouts, the artist who designed it, succeeded extremely well in demonstrating how beautiful staining can be.